

# Crystal Structure of Unligated Guanylate Kinase from Yeast Reveals GMP-induced Conformational Changes

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**Introduction:** Guanylate kinase (GK) catalyzes the reversible phosphoryl transfer from ATP to GMP in the presence of  $Mg^{2+}$ . GK plays important roles in the synthesis of nucleotide precursors and in the cGMP cycle. This enzyme may also be involved in guanine nucleotide-mediated signal transduction pathways by regulating the GTP:GDP ratio. Human GK is important in the activation of the anti-herpesvirus drugs acyclovir and gancyclovir and the anti-HIV agent carbovir. Like other NMP kinases, GK consists of three dynamic domains: the CORE, LID, and NMP-binding domains. GK is unique among NMP kinases in that its NMP-binding domain consists of a four-stranded  $\beta$ -sheet and only a short helix as revealed by X-ray crystallography, whereas the NMP-binding domains of other NMP kinases are all  $\alpha$ -helical.

**Methods and Materials:** Single crystal X-ray diffraction.

**Results:** The crystal structure of GK from yeast with a non-acetylated N terminus has been determined in its unligated form (apo-GK) as well as in complex with GMP (GK•GMP). The structure of apo-GK was solved with multiwavelength anomalous diffraction data and refined to an *R*-factor of 0.164 ( $R_{\text{free}} = 0.199$ ) at 2.3 Å resolution. The structure of GK•GMP was determined using the crystal structure of GK with an acetylated N terminus (Stehle & Schulz, 1992, *J. Mol. Biol.* **224**, 1127-1141) as the search model and refined to an *R*-factor of 0.156 ( $R_{\text{free}} = 0.245$ ) at 1.9 Å. Dramatic movements of the GMP-binding domain and smaller but significant movements of the LID domain have been revealed by comparing the structures of apo-GK and GK•GMP. Apo-GK has a much more open conformation than the GK•GMP complex. Systematic analysis of the domain movements using the program DynDom (Hayward & Berendsen, 1998, *Proteins*, **30**, 144-154) shows that the large movements of the GMP-binding domain involve a rotation around an effective hinge axis approximately parallel to helix 3, which connects the GMP-binding and CORE domains. The C-terminal portion of helix 3, which connects to the CORE domain, has strikingly higher temperature factors in GK•GMP than in apo-GK, indicating that these residues become more mobile upon GMP binding.

**Conclusions:** The results suggest that helix 3 plays an important role in domain movement. Unlike the GMP-binding domain, which moves toward the active center of the enzyme upon GMP binding, the LID domain moves away from the active center and makes the presumed ATP-binding site more open. Therefore, the LID domain movement may facilitate the binding of MgATP. The structure of the recombinant GK•GMP complex superimposes very well with that of the native GK•GMP complex, indicating that N-terminal acetylation does not have significant impact on the three-dimensional structure of GK.

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